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Undertakings : Intellectual Property Laws, Patents, Trademarks, Designs, Copyrights, Licencing, [redacted]stigations, Litigations
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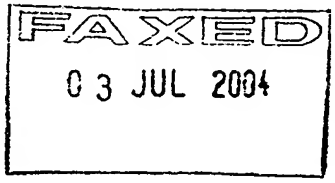

Via FAX/Courier
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July 3, 2004

Dear Sirs

Re : **RESPONSE TO THE WRITTEN OPINION UNDER RULE 66.3**
PCT International Application No. PCT/IN03/00289
dated 27 August 2003 (27.08.2003)
Applicant : LUPIN LTD. et al
Title : Herbal Extract Comprising A Mixture Of Saponins Obtained From Sapindus Trifoliatus For Anticonvulsant Activity.
Priority Date : 28 August 2002 (28-08-2002)
Agent's File Reference : FPAA335PCT

Please refer to the Written opinion dated June 7, 2004 issued on the above. With regard to the objection of anticipation based on Chaturvedi et.al (XP0090202733) and that of obviousness based on EP 0767177, Patent abstracts of Japan, cited in the International Search Report, the applicants disagree with the Opinion and submit the following arguments:

Chaturvedi et.al., XP 009027233 (D1) cited in the ISR and written opinion under "X" category teaches administration of respective triterpenoids at a concentration/dose of 40mg/kg and 100 mg/kg body weight of the mice tested for study of the anti-inflammatory and anticonvulsant activities respectively. From the description given in Paragraph 3, page 201; Paragraph 1, page 203; and from Table-I, page 205 of D1 it could be seen that hederagenin and hederagenin methyl ester acetate administered at a dose of 40 mg/kg exhibit 48.3 and 32.3 % anti-inflammatory activity whereas when they are administered at a dose of 100 mg/kg exhibit 30 and 20% anticonvulsant activity.

From Table-I, page 205 and the description given in Paragraph 1, page 203 of D1 it could be seen the route of administration of the triterpenoids to the test animals for evaluating the anticonvulsant activity is intraperitoneal.

The anticonvulsant activity of the triterpenoids were determined against pentylenetetrazol (PTZ) induced seizures in albino mice as mentioned in lines 1-2, page 203 of D1.

Further no mechanism of action of the triterpenoids in producing the anti-inflammatory/anticonvulsant activity has been discussed.

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In summary, document D1 relates to study of the anti-inflammatory and anticonvulsant activities of triterpenoids, specially hederagenin and hederagenin methyl ester acetate in albino mice administered through intraperitoneal. route at a dose of 100 mg/kg of body weight of the said animals, the said anticonvulsant activity determined against pentylenetetrazol (PTZ) induced seizures in albino mice. Further, no mechanism of action of the triterpenoids in producing the anti-inflammatory/anticonvulsant activity has been discussed.

The present Application No. PCT/IN03/00289 on the other hand, relates to a pharmaceutical composition comprising an aqueous extract of the pericarp of the fruit of the plant *Sapindus trifoliatus* and pharmaceutically acceptable additives, which exhibits binding affinities for receptor sites like GABA-Agonist, Glutamate-AMPA, Glutamate-Kainate, Glutamate-NMDA agonistic, Glutamate-NMDA glycine (strychnine intensive), and sodium channel (site 2) sites, which are known to have major mediatory role in anticonvulsant activity.

As would be evident from the discussion mentioned in pages 15-17 of present specification the pericarp of the fruit of the plant *Sapindus trifoliatus*, which constitutes 62 % of the fruit is made up of mainly glucose, saponins and primary metabolites. The extract of the pericarp of the fruit of the plant also shows the presence of the principal saponins, glucose and primary metabolites as evidenced by TLC and HPLC, which has been isolated and characterized as to contain a mixture of at least six hederagenin derivatives, all arising from single aglycon, viz. hederagenin. The concentration of hederagenin in the extract has been determined to be between 4 to 8% w/w of the extract. Further, the aqueous extract as present in the pharmaceutical composition of the present invention contains hederagenin in a range of between 0.001 to 1.00% w/v of the composition. Such an extract despite having a considerably low concentration of hederagenin exhibits a vastly superior anticonvulsant activity compared to that exhibited by pure hederagenin at a considerably high dose as disclosed in document D1. The ED_{50} of the extract of the present invention is only 7.72 mg/kg as against that of 100 mg/kg as reported in document D1, which is approximately $1/13^{th}$ of the later. Further, the route of administration of the extract of the present invention is nasal, whereas the route of administration in document D1 is intraperitoneal.

In addition, the anticonvulsant activity of the extract of the present invention was indicated only when it was evaluated in an *in vitro* animal model employing the Maximal Electroshock Seizure (MES) method, whereas the extract had no protective effect or in other words showed no anticonvulsant activity when evaluated employing the pentylenetetrazol (PTZ) induced seizures in albino mice as disclosed at page 21 of the present specification unlike that in document D1.

It is thus evident that the present invention provides an extract of the pericarp of the fruit of the plant *Sapindus trifoliatus* containing various components/active principles which in combination act in a synergistic way and exhibit anticonvulsant activity which is vastly superior to that of pure hederagenin and hederagenin methyl ester acetate disclosed in document D1 despite containing one of the active ingredient, viz. hederagenin in a considerably low concentration that prescribed in D1.

In essence, a thirteen fold increase in anticonvulsant activity using the extract of the present invention and that too utilizing a considerably low concentration of hederagenin is "unexpected" form the teachings of document D1 and hence not obvious or anticipated.

The present invention resides in providing a pharmaceutical composition containing the extract of the hederagenin and hederagenin methyl ester acetate as a mixture of various components/active principles which exhibits binding affinities for receptor sites like GABA-Agonist, Glutamate-AMPA, Glutamate-Kainate, Glutamate-NMDA agonistic, Glutamate-NMDA glycine (strychnine intensive), and sodium channel (site 2) sites, and is found to have vastly superior anticonvulsant activity . The binding affinities exhibited by the extract provides a safe prophylactic treatment for migraine, which too is not taught by the cited art.

It is further submitted that there is no mention of the *S. trifoliatus* pericarp, which is used in the present invention in the cited art D1. Neither is there mention of the binding affinities of the extract as indicated and claimed in your invention. It is further submitted that the present invention relates to extract from *S. trifoliatus* pericarp for nasal administrtion having specific binding site which may be used for prophylactic treatment of migraine mediated through anticonvulsant route which is neither taught nor motivated from this cited art. There is nothing in the cited art which teaches such plant extract from *S. trifoliatus* pericarp with specific binding activity which would be appropriate as an anticonvulsant pharmaceutical composition particularly for treatment of migraine. Accordingly, the present invention with the said extract at a level of 0.001 to 1% and defined binding affinities ought not to be regarded as anticipated by the cited art.

Regarding EP 767177 (D2) cited as 'Y' category citation, it is submitted that the same relates to treatment of nephritis. Technically, nephritis suggests a non-infectious inflammatory process involving the nephron. In general, nephritis is produced by antigen-antibody complexes (or some other unknown mechanism) trapped in the renal parenchyma. It teaches a pharmaceutical composition comprising hederagenin or its pharmaceutical acceptable salt or a solvate thereof for treatment of nephritis. The hederagenin used is extracted from *S. mukorosi* and is then purified by several methods. Its various derivatives, salt and solvates have been used in pharmaceutical compositions for treatment of nephritis. This thus teaches hederagenin extracted from plant *S. mukorosi* to have inhibitory effect on mesanglial cell proliferation.

On the other hand, the present invention relates to pharmaceutical composition with defined level of the hederagenin in the extract from S. trifoliatus for treatment of migraine through anticonvulsant route. This cannot be reached with the knowledge from this cited art. The cited art cannot motivate an ordinarily skilled person to conceive a pharmaceutical composition comprising extract from another plant source to act as anticonvulsant and for treatment of migraine through anticonvulsant route. It is further submitted that the teaching of anti-inflammatory effect of hederagenin extracted from plant *S. mukorosi* cannot be extended to the present invention as the treatment of migraine in the present invention is not brought about by anti-inflammatory effect but by the anticonvulsant route which is neither taught nor motivated by the cited art.

Moreover the present invention has nothing to do with nephritis in particular which is the main teaching of this cited art.

As to the cited art (Patent Abstract of Japan) as "Y" category citation, it is submitted that the same relates to dentifrice composition having inflammatory effect to prevent gum periodontitis comprising extract containing hederagenin extracted from plant *S. mukorosi*. The present invention relating to of pharmaceutical composition with anticonsulvant activity comprising extract of defined level of the hederagenin in the extract from *S. trifoliatus* for treatment of migraine through anticonvulsant route cannot be reached with the teachings of this cited art. The cited art alone or in combination may lead one to try other plant extracts for anti-inflammatory activity. But to reach to the present invention with extract of another plant having defined level of hederagenin and with defined binding affinities involves inventive step, more so to be effective for treatment of migraine via anticonvulsant route. None of the cited art thus can be regarded as rendering the present invention as obvious.

The above may please be taken into consideration while establishing the International Preliminary Examination Report. In the instance, however, any further clarification from the applicant be needed by the Examining Authority, a further Written Opinion may kindly be issued to enable the applicants to have the opportunity to clarify any doubts to facilitate appreciation of the invention and the claims.

Yours faithfully,

Ganguli

Dr. Sanchita Ganguli
Applicant's Agent